This application claims the benefit of priority under 35 U.S.C. §119 of PCT/US97/16017, filed on September 10, 1997 which is a continuation-in-part of U.S.S.N. 08/711,568 filed on September 10, 1996, now U.S. Patent No. 5,856,462.

REMARKS

The above amendment introduces no new matter. Claims 1 and 3-5 are pending and stand rejected. Applicants gratefully acknowledge that the Examiner has found convincing certain of Applicant's arguments in their response filed October 9, 2002. Accordingly, rejections of record not reiterated in the present Office Action have been overcome.

Priority

The Office Action states that Applicants are required to amend the first line of the specification to indicate the relation of the instant application (U.S.S.N. 09/103,745) to the parent applications if the benefit of the filing date of the prior application is sought (35 U.S.C. 120 and 37 CFR 1.78). Accordingly, Applicants have amended the first line of the specification as requested by the Examiner to state the complete basis of priority and the relation of the two priority applications.

Double Patenting

The Office Action acknowledges Applicant's prior commitment to file a Terminal Disclaimer, disclaiming any term of the instant patent beyond the expiration of U.S. Patent No. 5,856,462, upon a finding by the Examiner that the pending claims are otherwise patentable. Accordingly, pending such a finding of allowable subject matter, Applicants respectfully request that the requirement for filing such a Terminal Disclaimer continue to be held in abeyance.

Rejection under 35 U.S.C. §102(a)

The Office Action states that claim 1 has been rejected under 35 U.S.C. § 102(a) as being anticipated by Krieg (WO/962555 or Krieg et al. (1996) Antisense & Nucleic Acid Drug Development 6: 133-9) or Zao et al. ((1996) Biochemical Pharmacology 51: 173-182) "for the same reasons of record as set forth in the Office Action of September 9, 1999." The Office Action further states that, despite Applicant's amendment to claim 1, "the claims as written are

still broad enough to encompass the cited prior art....all the above references teach an inverted modified phosphorothioate CpG motif." Applicants respectfully note that, with regard to these references, the Office Action of September 9, 1999 states that "each....teach CpG oligos as claimed." Applicants respectfully submit that the cited references fail to anticipate the pending claims under 35 U.S.C. §102(a) for the reasons which follow.

First, with regard to the Zao et al. reference, Applicants respectfully note that this reference, which was co-authored by the inventor of the instant invention, Sudhir Agrawal, was overcome as anticipatory prior art by a Declaration under 37 C.F.R. §1.132 in the related priority application 08/711,568 (now U.S. Patent No. 5,856,462) (see attached Exhibit A). As that Declaration, signed by Sudhir Agrawal, stated broadly that "I am the sole inventor of the subject matter which is disclosed in this publication and disclosed and claimed in the (08/711,568) patent application," it effectively rebuts the assertion that the Zao et al. publication represents knowledge or use "by others" in this country or description in a printed publication before the invention thereof by the applicant for patent. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §102(a) in view of the Zao et al reference.

Applicants further believe the rejection under 35 U.S.C. §102(a) in view of Krieg (WO 96/02555) and Krieg et al. ((1996) Antisense & Nucleic Acid Drug Development 6: 133-139) should be withdrawn for the reasons that follow. As with the Zao et al. reference, the Krieg WO 96/02555 reference was addressed during prosecution of the priority application 08/711,568. Applicants' Supplemental Response in that application dated June 1, 1998 notes that "In a Written Opinion in the PCT counterpart of the above-identified case, it was pointed out that reference WO 96/02555, currently of record, discloses an oligonucleotide containing methylcytosine CpG at page 8, third paragraph....(and)...claim 1 has been amended and claim 8 has been cancelled to remove the subject matter disclosed in that reference."

Like claim 1 in priority application 08/711,568, the instant claim 1 does not include modified 5-methylcytosine modified CpG-containing phosphorothioate oligonucleotides. Furthermore, this reference does not teach "an inverted modified phosphorothioate CpG motif" as stated by the Office Action. The specification defines an inverted CpG as "a 5'-GpC-3'

dinucleoside" (at page 11, line 4). Furthermore the claimed subject matter encompasses an "oligonucleotide that is complementary to a portion of a genomic region or gene for which inhibition of expression is desired, or to RNA transcribed from such a gene." When read together, the claimed subject matter requires the modification of a CpG antisense sequence, which is complementary to a portion of a genomic region or gene or to RNA transcribed from such a gene, to correspond to an inverted CpG (i.e. GpC) sequence. Notably, the Krieg reference does not teach inversion of antisense CpG sequences, which is distinct from, for example, the fortuitous occurrence of a GpC sequences within some of their reported oligonucleotides. at page 134, 1st paragraph of second column).

Furthermore, the Krieg (WO 96/02555) reference predominantly teaches immunomodulatory oligonucleotides that do not involve antisense effects. Indeed, this Krieg reference states that the investigated oligonucleotides "showed no greater homology" to sequences in GenBank than non-stimulatory oligonucleotides and, further, that "no hybridization to Northern blots..." was detected with the investigated oligonucleotides (at page 12, lines11-19). The same reference makes passing mention of "Therapeutic Uses for Neutral Oligonucleotides" (i.e., non-immunostimulatory oligonuleotides at page 21, lines 28-34) including their use as antisense oligonucleotides. Notably, "neutral oligonucleotides" are defined as oligonucleotides that do not contain an unmethylated CpG or, preferably, oligonucleotides which contains a methylated CpG dinucleotide – preferably 5' methyl cytosine CpG's (at page 10, lines 22-30). Accordingly, this passing mention of antisense-effecting oligonucleotides fails to anticipate the claimed oligonucleotides that do not include 5' methyl cytosine CpG modified oligonucleotides, and further, that require both a phosphorothioate linkage and a modified CpG that is an alkylphosphonate CpG, an inverted CpG, a 2'-O-substituted CpG, a stereospecific phosphorothioate CpG, a phosphotriester CpG, a phosphoramidate CpG, or a 2'-5' CpG.

Therefore the Krieg reference fails to provide a specific teaching containing every limitation of the claimed invention. As anticipation under 35 U.S.C. § 102(a) requires that every limitation of the claim be present in the asserted reference, Applicants note that Krieg (WO 96/02555) fails to anticipate the claimed invention and, accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Applicants further note that the Krieg et al. reference ((1996) Antisense & Nucleic Acid Drug Development 6: 133-139), like Krieg (WO 96/02555), teaches oligonucleotide modifications that affect the magnitude of B cell stimulation by CpG motifs, i.e., non-antisense effects. Furthermore, this reference does not teach compositions having all of the limitations of the claimed invention. In particular, the reference does not teach antisense CpG oligonucleotides carrying both phosphorothioate linkages and a a modified CpG that is an alkylphosphonate CpG, an inverted CpG, a 2'-O-substituted CpG, a stereospecific phosphorothioate CpG, a phosphotriester CpG, a phosphoramidate CpG, or a 2'-5' CpG. Indeed, the Krieg et al. reference does not teach inversion of a CpG dinucleotide that is complementary to a gene or to an RNA transcribed from such a gene (as distinct from fortuitous GpC sequences occurring within an antisense oligonucleotide). Furthermore Applicants note that the methylphosphorothioate (MPS) oligonucleotides of Krieg et al. carry both a methyl group and a thioate on the same internucleoside phosphorous. In contrast, the claimed invention includes alkylphosphonate phosphorothioate oligonucleotides in which the modified CpG "is a CpG dinucleoside in which the C nucleoside and the G nucleoside are covalently linked to each other through an alkylphosphonate internucleoside linkage" (emphasis added, see page 10, lines 24-27). Therefore the instant claimed oligonucleotides do not encompass the methylphosphorothioate (MPS) chemical species of the Krieg et al. reference.

Accordingly, reconsideration and withdrawal of the rejection under §102(a) in view of Krieg et al. ((1996) Antisense & Nucleic Acid Drug Development 6: 133-139) is respectfully requested.

Rejection under 35 U.S.C. §112, first paragraph (enablement)

The Office Action opines that "the specification, while being enabling for methods of using the contemplated compounds in cell culture, does not reasonably provide enablement for methods of treating mammals or methods of therapy using the instantly contemplated compounds." This aspect of the rejection is repeated at the end of the Office Action, where it is stated that the examples in the instant application provide an "*in vitro* model" that is not predictive of the claimed "*in vivo*" methods because of various cited problems occurring *in vivo* such as interactions with plasma and/or cellular proteins (page 9, 2nd paragraph).

Applicants respectfully disagree with this assessment of the specification and point to Examples 2-4 (at pages 18-20) of the instant application which teach intravenous injection of modified CpG-containing phosphorothioate oligonucleotides whereby the specific modifications reduce *in vivo* splenomegaly (Example 2), reduce *in vivo* platelet depletion (Example 3) and reduce serum alanine aminotransferase (ALT) and/or serum aspartate aminotransferase (AST) stimulation (Example 4) in whole animals (i.e. CD-1 mice and Fischer rats). Furthermore, the Office Action cites the Agrawal reference ((1996) Trends in Biotechnol. 14: 376-87) in support of its rejection for lack of enablement, but the Agrawal reference states that "it is clear from some of the studies mentioned in this review and many other published reports that PS-oligonucleotides show more sequence-specific antisense activity in animal models than in cell culture experiments" (at page 384, 1st paragraph of Conclusions section). Therefore, one of the references cited to support the enablement rejection actually supports enablement of anitsense *in vivo* in whole animals. Accordingly, reconsideration and withdrawal of this aspect of the rejection is respectfully requested.

The Office Action further states that claims 3-5 have been rejected under 35 U.S.C. §112, first paragraph, based upon quotations from various antisense review articles that are said to provide a "comprehensive analysis of the state of *in vivo* anti-sense mediated gene inhibition." In particular, while not rebutting the credibility or relevance of the "primary literature" previously submitted by the Applicant in evidence of enablement, the Office Action opines that the cited reviews are more indicative of the overall state of the art.

Applicants respectfully traverse this enablement rejection in view of the cited portions of each of these review articles. In particular, Applicants note that the cited portions of each of these articles is neither representative of the overall teachings of the review nor reflective of enablement of the instant claimed invention. Each reference is addressed below.

First, the Office Action states that "a recent (2002) article by Braasche et al. ((2002) Biochemistry 41: 4503-4510)...(indicates that)....'gene inhibition by antisense oligomers has not proven to be a robust or generally reliable technology...(and that)...many researchers are skeptical about the approach, and it has been suggested that many published studies are at least partially unreliable.'" Applicants respectfully note that the cited quote from Braasche et al. is

not representative of the overall teachings of the review article. In particular, the Braasche et al. reference also states that "Oligomers with improved chemical properties, combined with advances in cell biology and success in clinical trials are affording powerful new options for basic research, biotechnology, and medicine" (Abstract, at line 7-9). The Office Action further cites the Braasche et al. reference for the proposition that access of antisense oligonucleotides to mRNAs is unpredictable - often being blocked by secondary structure of the RNA. Applicants note that the same reference states, with regard to this problem, "it may be necessary to screen 20 or more oligomers before identifying one that functions adequately" (at page 4503, 2nd paragraph). Applicants note that there is no evidence in the record that screening "20 or more oligomers," while possibly inconvenient, is anything more than routine in the art and not grounds for claiming a lack of enablement. This same argument is relevant to the cited portion of the Gerwitz et al. reference ((1996) Proc. Natl. Acad. Sci. USA 93: 3161-63). Indeed, Applicants have already provided a reference (Milner et al. (1997) Nature Biotechnology 15: 537-41) that discloses a high throughput screen to identify antisense inhibitors – evidencing enablement of the claimed invention by routine methods known in the art. Still further with regard to Braasche et al., Applicant note that the cited portions are loosely based upon old studies that did not utilize the improved modified CpG phosphorothioate oligonucleotides of the instant invention. For example, the Office Action states that Braasche et al. cites "toxicity and immunological problems caused by antisense oligos" as supporting "the unpredictable efficacy of antisense compounds *in vivo*." However, it is precisely these unwanted side effects that the instant invention is directed to avoiding. Accordingly, the cited statements do not support lack of enablement of the instant claimed gene modulatory methods and reconsideration and withdrawal of the rejection is respectfully requested.

The Office Action further cites the Agrawal reference ((1996) Trends in Biotechnol. 14: 376-87) for the proposition that it is "difficult to generalize that all oligonucleotides are taken up in all cells with the same efficiency" (quoting from page 378). Applicants first note that, even if variability in uptake does occur between individual species of antisense oligonucleotide, individual optimization of the dose and means of delivery of the claimed antisense oligonucleotides is within the routine skill in the art. Applicants further note that the cited portion of the Agrawal reference is discussing all oligonucleotides of every imaginable sequence and with every known chemical modification, and not the specifically claimed modified CpG-

containing phophorothioate antisense oligonucleotides of the instant invention. Accordingly, the cited portion does not support unpredictability in uptake in support of a lack of enablement of the claimed antisense oligonucleotides. Still further, Applicants note that the cited portion of Agrawal misrepresents the overall teachings of this reference, the complete abstract of which reference states:

> "Antisense oligonucleotides have the ability to selectively block disease-causing genes, thereby inhibiting production of disease-associated proteins. The specificity and application of antisense oligonucleotides have been strongly validated in animal models for various disease targets. Based on the pharmacological, pharmacodynamic and pharmacokinetic profiles, the first generation of antisense oligonucleotides – phosphorothioates – have reached the stage of human clinical trials for various diseases. While ongoing human clinical trials are being carried out to further establishing the safety and efficacy of these oligonucleotides, the experience gained is providing a basis for designing a second generation of antisense oligonucleotides."

Accordingly, the portion of Agrawal cited in the Office Action is not representative of the overall teachings of this reference, which overall strongly supports enablement of antisense technology. Indeed, the Agrawal reference, published by the inventor, further discusses improvements provided by the features of the modified antisense oligonucleotides of the claimed invention – thereby strongly supporting enablement of the instant invention (see page 386).

Finally, the Office Action cites portions of Branch ((1998) TIBS 23: 45-50) which "discuss the non-specific toxicity effects of *in vivo* antisense administration," and further cites Branch's teaching that "non-antisense effects are not currently predictable, rules for rational design cannot be applied to the production of non-antisense drugs...(and)...these effects must be explored on a cases by case basis." The Office Action further cites Tamm et al. ((2001) The Lancet 358: 489-97) for the premise that "immune stimulation is widely recognized as an undesirable side-effect (of antisense oligonucleotides, and)....the immunostimulatory activity of a phosphorothioate-modified oligonucleotide is largely unpredictable and has to be ascertained experimentally." Applicants respectfully point out that the cited portions of Branch and Tamm et al. do not support lack of enablement of the instant invention, because these portions relate to a discussion of the non-antisense (e.g., immunomodulatory) activities of such oligonucleotides.

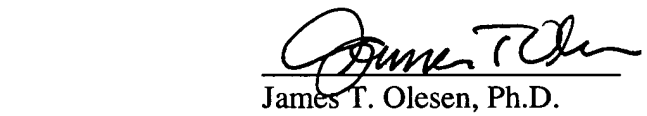
In contrast, the instant invention is directed to antisense methods for modulating gene expression in a mammal with reduced side effects. Indeed, it is an object of the instant invention to reduce non-antisense side-effects of antisense compositions and the instant application demonstrates advances in antisense technology in precisely this area. Accordingly, the cited portions of Branch and Tamm et al. do not relate to the compositions of the instant invention and reconsideration and withdrawal of the rejection in view of Branch and Tamm et al. is respectfully requested.

CONCLUSION

Applicant believe that the presently maintained rejections of the pending claims have been fully overcome by the amendment and arguments presented above. Accordingly, Applicant respectfully submits that the pending claims are in condition for allowance, and prompt acknowledgment of such is respectfully requested. If the Examiner believes that any further discussion of this communication would be helpful, he is encouraged to contact the undersigned by telephone.

No additional fees are believed to be due in connection with this communication, however, please apply any additional charges, or credit any overpayment, to our Deposit Account No. 08-0219.

Respectfully submitted,
HALE AND DORR LLP



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Date: April 25, 2003

EXHIBIT A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Atty Docket No. 47508-642 ; Client Docket No. 227.0 US)

Serial No.:	08/711,568	)	
Filed:	10 September 1996	)	**Group Art Unit:** 1635
Inventors:	Sudhir Agrawal	)	**Before the Examiner:**
Title:	METHOD FOR USING OLIGO-	)	John L. LeGuyader
	NUCLEOTIDES HAVING MOD-)		
	IFIED CpG DINUCLEOSIDES	)	

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Hon. Assistant Commissioner for Patents:

I, SUDHIR AGRAWAL, herby declare that:

I am the sole inventor of the subject matter described and claimed in United States Patent Application Serial No. 08/711,568, filed September 10, 1996, entitled METHOD FOR USING OLIGONUCLEOTIDES HAVING MODIFIED CpG DINUCLEOSIDES, for which this declaration is submitted.

I am a co-author of a report in Biochemical Pharmacology, Volume 51, pages 173-182 (1996). I am the sole inventor of the subject matter which is disclosed in this publication and disclosed and claimed in the above-identified patent application.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willlful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: Sudhir Agrawal

Dated: August 11, 1998